Factors affecting trap catch in pheromone-based monitoring of saddle gall midge Haplodiplosis marginata (Diptera: Cecidomyiidae)

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DOI: https://dx.doi.org */10.1002/ps.4721*

Rowley, C., Cherrill, A.J., Leather, S.R., Hall, D.R. and Pope, T.W. 2017. Factors affecting trap catch in pheromone-based monitoring of saddle gall midge Haplodiplosis marginata (Diptera: Cecidomyiidae). *Pest Management Science.*

29 August 2017

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Running title

Factors affecting trap catch in pheromone-based monitoring of *H. marginata*

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ABSTRACT

BACKGROUND: Saddle gall midge, *Haplodiplosis marginata* (von Roser) (Diptera: Cecidomyiidae), is a pest of cereal crops in Europe. Outbreaks are difficult to predict and effective monitoring tools are required to ensure the effectiveness of pest management options. The female sex pheromone, (*R*)-2-nonyl butyrate, provides the basis of a highly effective lure for this insect. Here, we demonstrate how the success of this lure can be influenced by parameters such as trap location, lure age, and interference between traps fitted with these lures.

RESULTS: A pheromone lure containing (*R*)-2-nonyl butyrate attracted male midges for at least 9 weeks under field conditions. Pheromone-baited traps performed best when situated away from field margins and below the height of the crop. Interference between nearby traps was evident at distances less than 20 m.

CONCLUSION: The results here offer new insights into the behavioural responses of male *H. marginata* to the female sex pheromone and provide practical recommendations for the use of *H. marginata* pheromone traps in the field.

Keywords: Cecidomyiidae, IPM, interaction, height, (R)-2-nonyl butyrate, wheat, longevity

Headings

1 INTRODUCTION

Saddle gall midge, *Haplodiplosis marginata* (von Roser) (Diptera: Cecidomyiidae), is a pest of cereal crops in Europe that has exhibited a sporadic pattern of outbreaks for several decades. The species is univoltine, with adults emerging in May following a larval overwintering stage. After mating, *H. marginata* females oviposit on the leaves of cereal plants and wild grasses. Upon hatching larvae begin to feed on the stem of the host plant from beneath the leaf sheath. Larval feeding causes the formation of saddle-shaped galls on the stem which can affect plant development and cause yield loss.[1 - 3] Spring crops of wheat and barley are most at risk from this pest,[4,5] particularly where damage coincides with stem extension.[6] Regular crop rotations can reduce *H. marginata* numbers through removal of the host crop,[5] but as the overwintering stage can survive in the soil for several years the population may still persist.[7] The biology and ecology of this pest have been reviewed in detail in recent attempts to consolidate the existing information on this insect.[8,9] Such reviews have highlighted the need for more effective detection and monitoring tools given the sporadic and often inconspicuous nature of the pest. This is also of importance for application of chemical controls which need to be timed to coincide with the vulnerable egg-laying stage to be effective.[10,11] Currently, farmers and agronomists must regularly check the crop for adults and eggs which is time-consuming and risks missing the early stages of pest outbreaks.

Pheromone traps are regularly used for detection of pest species and their sensitivity means that insects can be detected even when population density is low, such as the onset of adult emergence.[12] The sex pheromones of pest species of gall midges are relatively well-studied [13] and have been successfully applied to in-field monitoring and detection in a range of species such as Hessian fly, *Mayetiola destructor* (Say);[14] orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin);[15,16] and apple leaf midge, *Dasineura mali* (Keiffer).[17,18] Pheromone monitoring of swede midge (*Contarinia nasturtii* (Keiffer)) has been recommended for use in combination with a predictive model to determine the time of emergence.[19] Censier et al.

(2014)[20] identified the major component of the female sex pheromone of *H. marginata* as 2-nonyl butyrate. More recently, an effective lure for this pest has been developed, based on the optimised blend and loading of pheromone and dispenser type.[21] For this information to be of practical benefit however, more information is needed on how best to deploy traps baited with these pheromone lures.

The longevity of a pheromone lure is dependent on the initial loading and the subsequent rates of release and degradation of the compound, which are in turn influenced by the pheromone dispenser and by environmental conditions such as temperature and UV light.[22] Ideally a lure should exhibit a constant rate of release and last for the duration of the insect flight season. In previous work, polyethylene vials loaded with 0.5 mg (*R*)-2-nonyl butyrate were identified as effective dispensers for the *H. marginata* pheromone, lasting for at least four weeks under laboratory conditions.[21] Here we determine the effectiveness of the lure over time under typical field conditions. This information has implications for catch interpretation and the need to refresh lures if they are in use over the entire flight period of *H. marginata*.

In the development of many pheromone trap systems, trap position has been found to have a considerable influence on trap catch.[23 - 25] Pheromones disperse in the form of plumes, which insects detect and follow upwind to the source of the odour. Pheromone plume structure and the ability of the insect to navigate to the source are both influenced by external factors such as wind speed and direction, landscape features, pheromone concentration, and signal interference from other sources. The positioning of a trap in relation to the surrounding environment and the insect itself is therefore of importance. Pheromone plumes of the same compound have been shown to interact causing disruption of the catch in a particular trap.[26] Given that several traps are often deployed within an area to increase confidence in the numbers caught, it is essential to know the minimum inter-trap distance at which interference occurs to ensure optimum catch in all traps.[27] This information would also help evaluate the suitability of this lure for use in mass trapping strategies if traps have a considerable range of attraction. *Haplodiplosis marginata,* like many Cecidomyiidae, are not thought to be strong flyers and may be particularly influenced by factors affecting the pheromone plume. We therefore aimed to determine the optimal positioning of *H. marginata* pheromone traps in relation to height, distance from the field margin and proximity to other traps.

2 EXPERIMENTAL METHODS

2.1 Field sites

Three sites with existing populations of *H. marginata* located in Oxfordshire (51°55"N, 1°10"W); Buckinghamshire (51°37"N, 0°48"W) and Wiltshire (51°2"N, 1°57"W) were used. Pheromone dispensers were placed in standard red delta traps (Agralan, Wiltshire, UK) containing a removable sticky insert (15 cm x 15 cm). Polyethylene vials (26 mm x 8 mm x 1.5 mm thick, Just Plastics Ltd., London, UK) containing (*R*)-2-nonyl butyrate (0.5mg; 98% enantiomeric excess) synthesised as described in Rowley et al., 2017,[21] were used as lures for all experiments. Traps were hung from fibreglass canes and positioned at the height of the ear of the wheat crop unless otherwise stated. Mean wind speed and direction data for each site were obtained by pooling data for the three nearest weather stations to each field site from the Met Office MIDAS dataset.[28] Adult *H. marginata* were identified based on antennal and genital morphology[29] and counted using a bifocal microscope. All statistical analyses were done in R 3.3.1.[30] Linear mixed effects models were fitted with the lme function from the nlme package[31] and post-hoc multiple comparisons (Tukey's Contrasts) were performed using the glht function from the multcomp package. Residual plots were used to check for violations of model assumptions.

2.2 Field experiments

2.2.1 Lure longevity

Traps were positioned in two fields of winter wheat: one each at the site in Oxfordshire and Buckinghamshire, between 3 May – 1 July 2016. Winter wheat growth stages were approximately 37 at the start of the experiment and 69 by the end.[32] Traps were positioned at the height of the ear along two parallel transects 20 m apart. Four traps were placed at intervals of 40 m along each transect. Traps placed at the same distance along the two transects represented a pair, each trap baited with either a pheromone lure that remained in the trap throughout the season or a lure that was replaced weekly. New lures were replaced on days 6, 13, 20, 29, 34, 43, 50 and 59 of the experiment at which time the sticky inserts of all traps were renewed and the positions of traps within a pair were switched to reduce positional effects. The height of traps was adjusted each week to match the growth of the crop. At the end of the experiment aged lures were retained. The remaining pheromone was extracted from each lure individually in hexane (5 ml) containing dodecyl acetate (1 mg) as the internal standard. Extracts were analysed by GC with FID on a capillary column (30 m x 0.32 mm i.d. x 0.125 μ film thickness) coated with DB5 (Agilent) with splitless injection (220°C) and the oven temperature held at 50°C for 2 min and then programmed at 10°C/min to 250°C. Data for the first two weeks of the experiment at the Bucks site and the first week at the Oxon site were removed due to low catches in all traps unduly influencing the model fit. Numbers of *H. marginata* caught per day for each trap were log(x+1) transformed to improve the homoscedasticity of the data. The effect of field, days elapsed and lure type (old or new) on catch were analysed using a linear mixed model with pair as a random effect and all other terms as fixed effects.

2.2.2 Trap height

Traps were positioned at the site in Oxfordshire between 13 – 19 May 2016 in two adjacent fields. One field was winter wheat and the other spring wheat, which were at growth stages 45-47 and 29-31 respectively over the experimental period. Traps were deployed in two 4 x 4 Latin squares, one in each field with at least 200 m between the two squares. Four height treatments were used, measured from the ground to the base of the trap: 0 cm, 40 cm, 80 cm and 120 cm. Treatment 0 cm was below the height of the crop in both fields. Treatment 40 cm was at the height of the ear in the field of winter wheat, and above crop height in the field of spring wheat. Treatments 80 cm and 120 cm were above crop height in both fields. Sticky trap inserts were renewed and trapped insects counted on day three and at the end of the experiment. Treatments within each Latin square were re-randomised on day three. Both sets of counts were used in the analysis. Numbers of *H. marginata* caught in each trap were log(x+1) transformed to improve the homoscedasticity of the data and were analysed using a two-way analysis of variance (ANOVA).

2.2.3 Distance from field margins

Traps were positioned at all three sites in fields of winter wheat between 19 May – 1 June 2016. The crop was at growth stage 47 at the start of trapping and 59 at the end. Three traps were positioned at 20 m intervals on a transect perpendicular to the field margin, with the first trap placed in the margin itself. Transects were placed on field margins of each aspect (north, south, east and west facing) in each field giving 12 transects in total. Each transect was later classified as upwind, downwind or crosswind according to the prevailing wind direction for the trapping period. Sticky inserts were changed weekly. Count data were pooled over the entire trapping period and the effects of trap position in relation to field, wind direction and distance from the field margin on catch were analysed using a linear mixed model with transect as a random effect and all other variables as fixed effects. Distance was treated as a categorical variable and multiple comparisons of means were used to test for significant differences in catch between traps at different distances from the field margin.

2.2.4 Range of interference

Traps were positioned in a field of winter wheat at each of the three sites between 1 – 22 June 2016. The crop was at growth stage 59 at the start of trapping and 65 at the end. In each field were positioned four hexagonal arrays of traps with an additional central trap, so that all traps were equidistance apart with at least 80 m between arrays.[33,34] Each array had a different inter-trap distance (treatment): 5 m, 10 m, 20 m and 40 m, with each treatment occurring once per field. The sticky inserts of all traps were changed three times at an interval of one week. On each occasion the treatments were re-randomised within each field. The design gave three replicates of each inter-trap distance at each timepoint. The central trap remained in the same location regardless of the inter-trap distance. The relationship between inter-trap distance and mean catch of the outer and central traps was analysed using a linear mixed effects model with array as a random effect and all other variables as fixed effects, with distance treated as a continuous variable. Significant outliers were found in both downwind traps of one of the 20 m arrays over one particular trapping period. As these traps were determined to be unduly influencing the fit of the models, it was decided that these should be removed prior to analysis.

3 RESULTS

3.1 Field experiments

3.1.1 Lure Longevity

Over the entire experimental period, traps baited with old lures caught fewer male *H. marginata* than traps baited with new lures ($F_{1,94}$=50.65, P<0.001) but the difference in catch between the two types of lure did not change significantly over time ($F_{1,93}$=0.91, P=0.34) (Fig. 1). There were clear differences between the numbers of insects caught in all traps at each field site ($F_{1,6}$=43.95, P<0.001) and fewer insects were caught in all traps as the experiment progressed ($F_{1,94}$=466.54, P<0.001) (Fig.1). Analysis of the old lures (*N* = 4) revealed that 39.4% ± 0.7 of the pheromone from site 2 (Bucks) and 36.1% ± 1.4 of the pheromone at site 3 (Oxon) remained in the lures after the 59-day trapping period. Mean air temperatures during this time were 13.43 ± 0.10°C and 13.36 ± 0.11°C at sites 2 and 3 respectively; with the maximum air temperature not exceeding 25°C at either site.

3.1.2 Trap Height

Catch numbers differed between heights ($F_{3,30}$ =110.33, P<0.001), and catches at 0 cm and 40 cm heights differed between fields ($F_{9,24}$ =5.78, P<0.001) (Fig. 2). This difference was accounted for by trap height in relation to crop height. Post hoc tests revealed that field 1 in spring wheat (crop height of approximately 10 cm) had far higher numbers of insects trapped at 0 cm than 40 cm ($P<0.001$). Field 2 in spring wheat (crop height of approximately 40 cm) had no difference in catches at these two heights and had a higher number of insects caught at 40 cm compared to field 1 ($P<0.001$). Both fields therefore showed high catches in traps positioned below crop height and low catches in traps positioned above crop height (Fig. 2). Numbers of male *H. marginata* caught in each field were nearly identical: 49% were caught in field 1 and 51% in field 2. The fewest insects were caught at 80 cm and 120 cm; catches at 0 cm and 40 cm accounted for 98.3% of the total 3,100 trapped over the period of the experiment.

3.1.3 Distance from field margins

The distance of the trap from the field margin had a significant effect on trap catch ($F_{2,22}=8.19$, $P<0.01$) (Fig. 3). Post hoc testing revealed lower catches in traps positioned in the field margin compared to those positioned 20 m ($P<0.05$) and 40 m ($P<0.001$) into the crop. There was no difference in catch between the traps placed 20 m and 40 m into the crop ($P=0.54$). Transect direction in relation to prevailing wind direction had no effect on catch ($F_{2,9} = 0.29$, $P=0.75$).

3.1.4 Range of interference

The number of male *H. marginata* caught per day in outer traps of the hexagonal array was higher compared to central traps ($F_{1,49}=22.58$, $P<0.001$) and was higher overall (all traps combined) in arrays with a greater inter-trap distance ($F_{1,6}=49.21$, $P<0.001$). Differences between the catch of outer and central traps reduced with increasing inter-trap distance ($F_{1,49}=12.93$, $P<0.001$) (Fig. 4).

4 DISCUSSION

The results presented here provide new insights into factors affecting the performance of pheromone-baited traps for *H. marginata* that will contribute to design of protocols for use of the traps for monitoring and potentially control of this pest.

Pheromone lures still attracted male *H. marginata* adults up to and including nine weeks in the field. This is comparable to similar commercially available lures for other pest species[35,36] and longer than the recommended usage time of six weeks for *Sitodiplosis mosellana* lures.[15,16] Lures replaced each week consistently caught more midges than lures maintained continuously, even at the beginning of the experiment. Release of pheromone from the polyethylene vials is first order, i.e. proportional to the amount remaining, and it seems unlikely that the small decrease in release rate during the first two weeks would have resulted in a significant decrease in catches. Lures for the experiment were stored in sealed aluminium foil bags, and it is possible that, after removal from the bags and installation in the traps, there was an initial "burst" of pheromone from the surface of the lures that may have given consistently higher catches during the first day.[37]

In the final week of trapping, old lures trapped 45% of the number of insects caught by new lures which is to be expected from the finding that 35 – 40% of the pheromone remained in the old lures at the end of the experiment. This concurs with earlier experiments where 6-week old field aged lures containing 1 mg of racemic 2-nonyl butyrate had 0.41 ± 0.02 mg of the compound remaining.[21] Thus these lures are likely to remain attractive over the entire flight period of *H. marginata*, which is typically 8 – 10 weeks.[8] This will reduce the cost and time required to operate this system in the field. There is some decrease in attractiveness during this period and further work is required to relate catches directly to population levels, but it is anticipated that population peaks may be reliably identified mid-season relative to catches in the previous weeks, alerting the farmer to a potential increase in oviposition activity.

The height of a pheromone trap relative to the height of the crop can strongly affect trap catch.[38] Cecidomyiidae are typically not strong fliers[39] and *H. marginata* appears to be no exception: the furthest flight distance recorded for males is just 120 m.[4] Cecidomyiidae males also tend to exhibit a lack of vertical movement during flight.[40] An earlier trapping study of *H. marginata* using passive traps placed at the same heights used in the present experiment found that 9.8 – 17% of males were caught in traps at 80 cm or above, compared to 25 – 33%

of females.[4] Here, we found traps at heights of 80 cm and above accounted for just 1.7% of the total insects caught. This may be a consequence of using active rather than passive traps, wind conditions during the experiments, and the absence of females caught as they generally fly at greater heights.[4,5] The effect of the lower trap heights varied between fields as a consequence of crop type. In the field of spring wheat, 92% of insects were caught at ground level which was the only trap below crop height. In the field of winter wheat only 43.7% of insects were caught at ground level while 54.4% were caught at 40 cm which was at the height of the ear of crop. As with most midge species, adult *H. marginata* are often found close to the soil surface as this is the location of emergence and mating.[5] The results indicate that the adults are relatively evenly dispersed within the crop, although not above, even though the pheromone plume from traps above the crop level would have extended into the crop. This is in contrast to results with several other midge species.[13] For apple leaf midge, catches in traps at 0.5 m above ground were only 30% of those at ground level, even though the canopy was much higher.[17] A similar effect of the interaction between habitat and trap height was observed in lesser grain borers (*Rhyzopertha dominica (F.)*) responding to an aggregation pheromone.[41] The presence of volatiles from the crop may also enhance mate seeking behaviour in this insect, as is the case with males of the brassica pod midge *Dasineura brassicae* (Winnertz).[42] A study of codling moth in orchards recommended that trap height be considered relative to the tree height rather than in absolute terms.[43] While the height of wheat crops may not vary to the same extent, this study supports the idea that the crop is important in standardising catches in monitoring traps between fields. Based on these findings, it would be most practical for farmers to position pheromone traps at the height of the ear, as is recommended for pheromone traps of *S. mosellana*.[44] This height not only gives a good level of performance but also makes them easier to find than traps placed at ground level.

Catches of *H. marginata* declined when pheromone traps were situated in field margins. Of the total number of insects caught, 22% were in the field margin traps compared to 35% and 43% caught in traps 20 m and 40 m into the field respectively. This result may be a function of the reduced area from which *H. marginata* could be attracted to the traps, given that most margins were not adjacent to areas with *H. marginata* populations. There were no differences in catch in traps positioned 20 m and 40 m into the crop, yet there was a trend towards increased catch with increasing distance from margins at two out of three study sites. The third site had the lowest catch with just 14% of the total insects trapped, which may account for the lack of a similar trend. Couch grass (*Elymus repens*) and other wild grasses have been shown to be excellent host plants for *H. marginata* [4,45] and weeds can increase pest populations by acting as alternate host plants[46]. The presence of weed species in field margins here did not appear to increase numbers of *H. marginata* in these areas, possibly because the in-field populations were substantial. Obstacles such as hedgerows and trees adjacent to the margins may have impeded dispersion of the pheromone plume and the flight of insects, but the direction of the transect in relation to wind direction had no effect on catch which suggests this was not the case. There were signs of predation on traps and, although not surveyed here, it is possible that natural enemy populations associated with the field margins could have affected *H. marginata* counts in these areas. Field margins can augment natural enemy populations in arable fields,[47] but any suppressive effect may be reduced with increasing distance into the crop.[48] In a study on European corn borer, *Ostrinia nubilalis* (Hübner) trap location, Derrick et al. suggest that in addition to increased catches, within-field trap placement is advantageous in that the uniform habitat of the crop results in a more reliable trapping system.[49] It is therefore sensible to propose that *H. marginata* pheromone traps should be placed in an open space in an area of the field with known populations to maximise insect capture. In practice, given that traps placed 40 m into the crop increase maintenance time with no appreciable gain in catch, we suggest that a position 20 m into the crop should be sufficient in most cases.

Female Cecidomyiidae have been shown to produce sex pheromones that act as attractants over long distances rather than eliciting short-range behavioural effects.[39] The high numbers of *H. marginata* caught in traps baited with (*R*)-2-nonyl butyrate support this however it raises the possibility of interference occurring between lures of nearby traps. The flight behaviour of *H. marginata* is not well studied, but *M. destructor* males exhibit plume following behaviour very similar to that of male moths when responding to female sex pheromones.[50] The range of interference within moth pheromone trap systems has been studied based on the idea that pheromone traps in the centre of an array of traps will catch fewer individuals than traps on the outer edges if plumes are interacting.[26,33,51,52] In the case of *H. marginata*, central traps caught fewer insects than the outer traps and this difference declined with increasing inter-trap distance. This indicates the occurrence of plume interactions, where the overlapping plumes from upwind lures divert the insect away from the central trap.[26,53,54] On this basis, trap interference appears to occur primarily at inter-trap distances below 20 m based on the model described here (Fig. 4). This should therefore be considered the minimum trap spacing to avoid pheromone plumes overlapping. There was also an overall reduction in catches in the traps with decreasing inter-trap spacing and it is conceivable that this resulted from a trapping out of insects in the area. Additional research is needed to relate trap catches and the potential for trap interference to *H. marginata* population densities. In a detection or monitoring trap it would be advantageous to use larger inter-trap distances where possible to avoid the possibility of interactions occurring at higher wind speeds. For mass trapping or pheromone disruption strategies, a minimum of 25 traps would need to be deployed per hectare to ensure coverage of the area at the current pheromone concentration. However, far higher catches can be obtained by increasing the pheromone loading to 2.5 mg or more.[21] Further research would be required to determine the minimum distance between traps at a higher pheromone loading but it is likely to be large enough to offset the increased pheromone production costs in order to get complete coverage over an area.

Future research into *H. marginata* pheromone traps should also focus on the relationship between trap catch and potential crop damage to provide farmers with vital information upon which to base pest management decisions. The recommendations for use presented here describe not only aspects of practical consideration for growers which are important in achieving reliable results from the product;[55] but also provide insight into the flight of male *H. marginata* following emergence and their responses to pheromone lures.

ACKNOWLEDGEMENTS

The authors are grateful to AHDB Cereals & Oilseeds for funding this work (project number 214-0002).

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Figure Legends

Figure 1. Catches of *Haplodiplosis marginata* males in traps baited with lures maintained continuously (old) or renewed at approximately weekly intervals (new) at two sites (3 May – 16 July 2016; *N* = 4 at each site; points show log counts, lines show results of model fit)

Figure 2. Mean catches (±SEM) of *Haplodiplosis marginata* males in traps positioned at different heights in fields of spring wheat (Field 1) and winter wheat (Field 2) at the Oxon field site (13-19 May 2016; *N* = 4 at each site and height; shaded areas represent traps at or below the height of the crop). Lowercase letters indicate significant differences between heights.

Figure 3. Mean catches (±SEM) of *Haplodiplosis marginata* males in traps positioned at increasing distance from the field margin (19 May – 1 June 2016; three sites, *N* = 4 at each site). Lowercase letters indicate significant differences between distances.

Figure 4. Interaction plot (± SE) from mixed effects model showing the interaction between trap location and inter-trap distance (lines). Mean catch of *Haplodiplosis marginata* males in central and outer traps in hexagonal arrays of different inter-trap distances at all sites (points) (1-22 June 2016; *N* = 3).

Figure 1

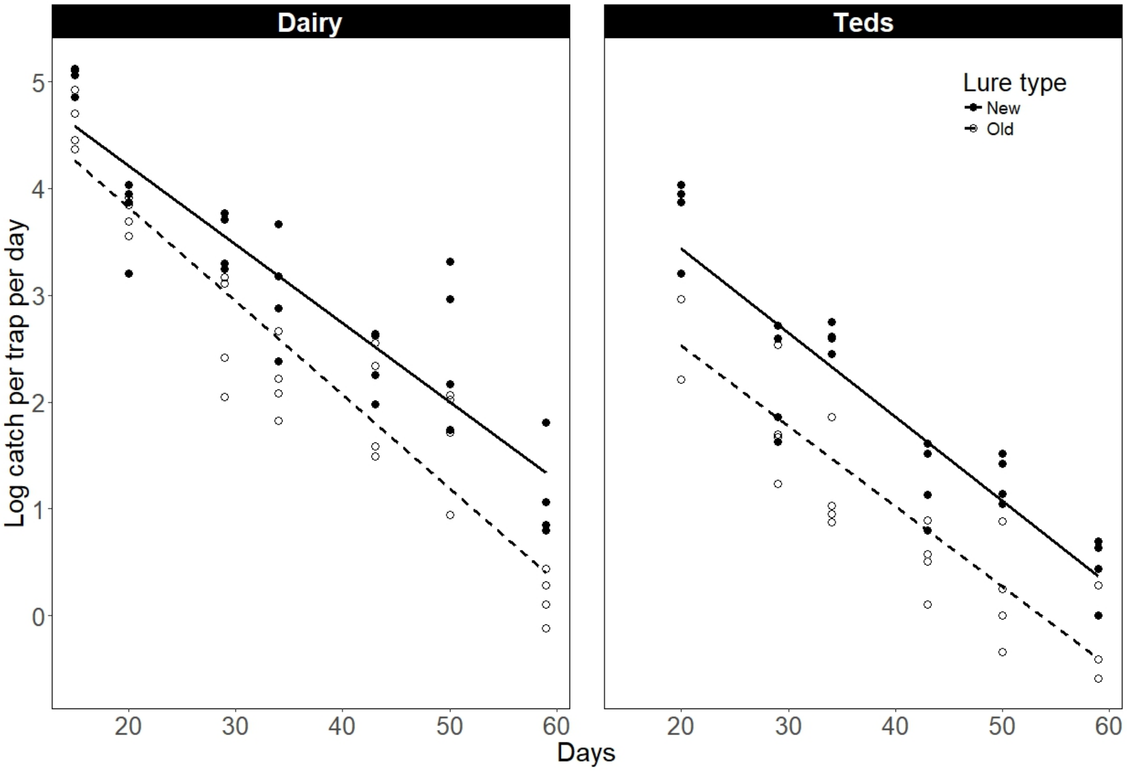

Dairy
Teds
Lure type
New
Old
Log catch per trap per day
0
1
2
3
4
5
20
30
40
50
60
Days

Figure 2

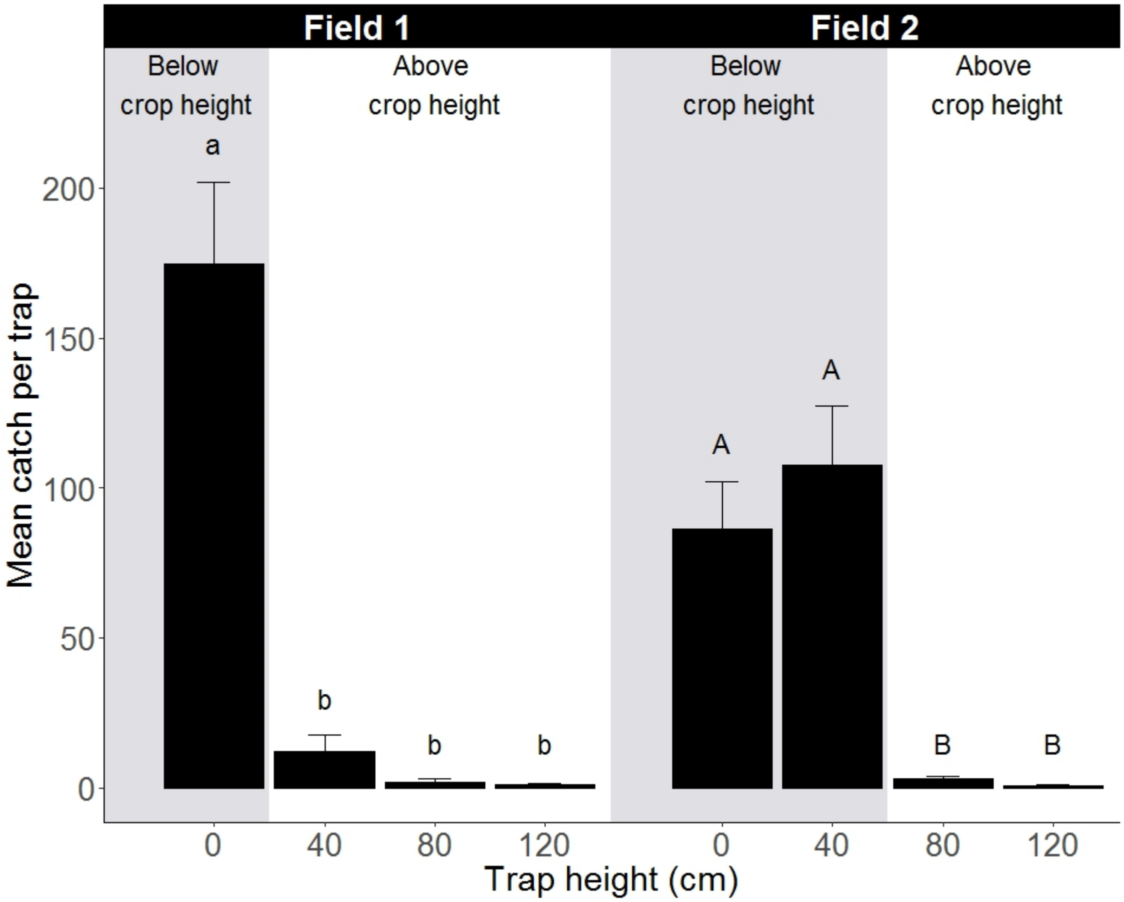
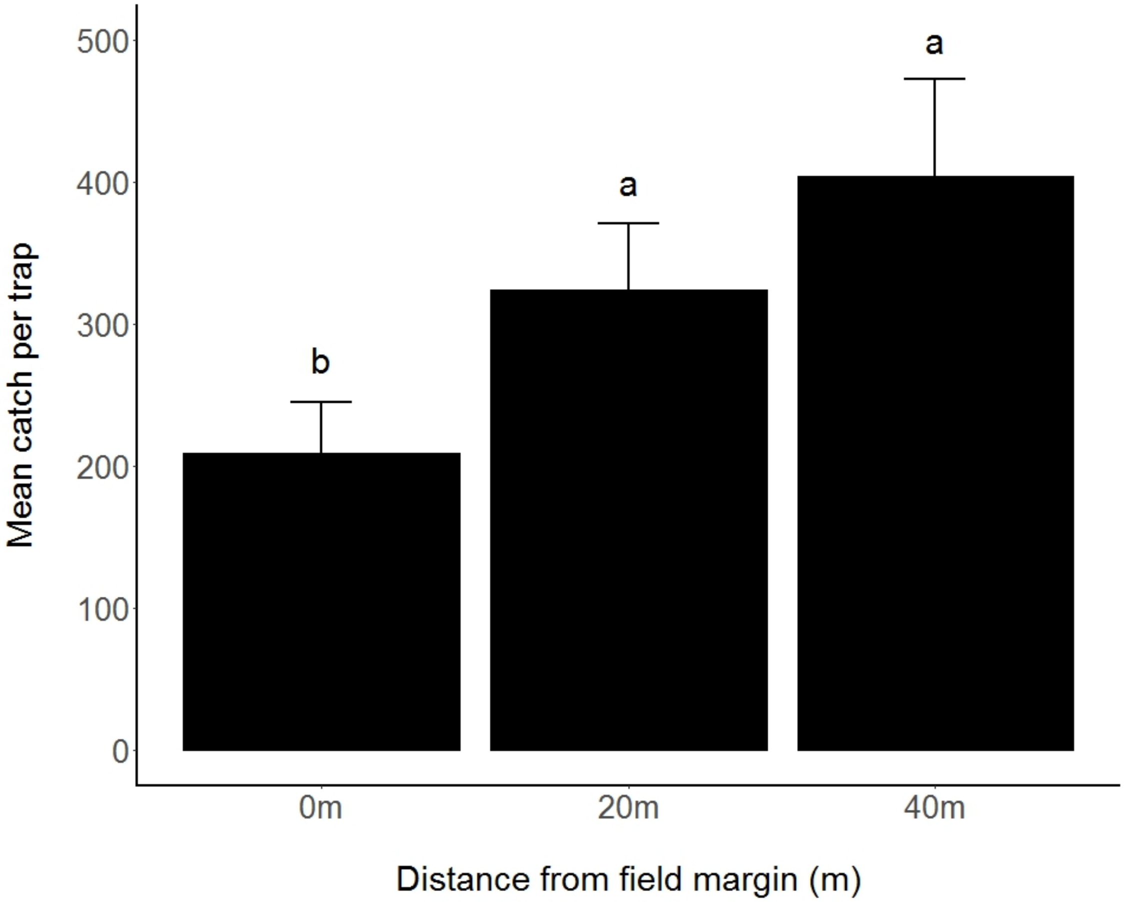
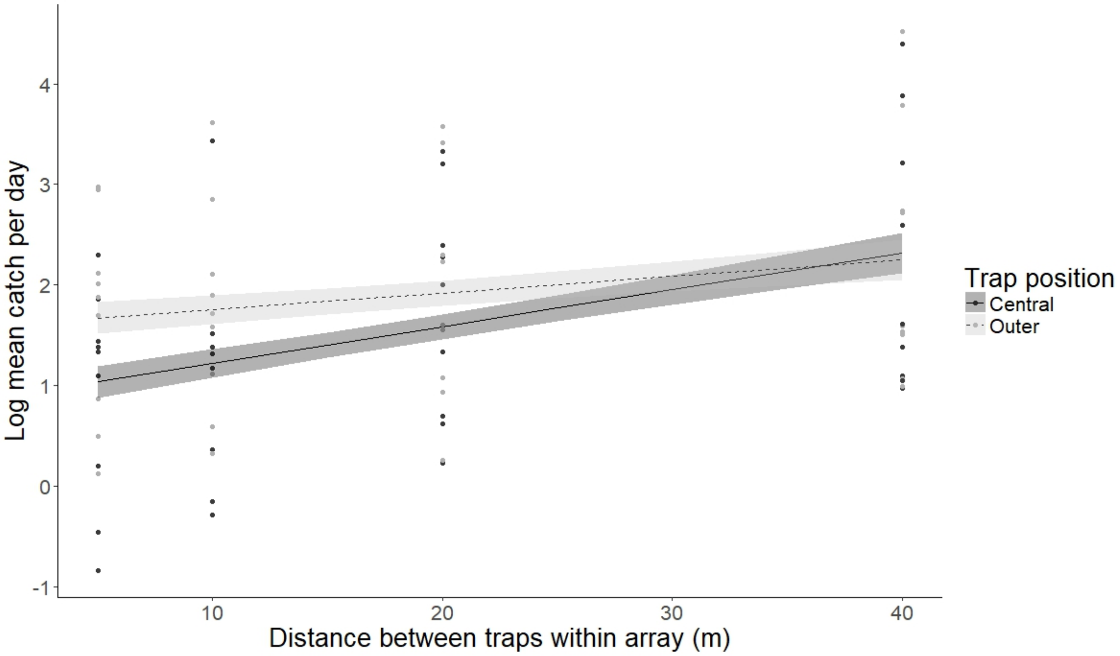

Field 1
Field 2
Below crop height
Above crop height
Below crop height
Above crop height
a
b
b
b
A
A
B
B
Mean catch per trap
0
50
100
150
200
0
40
80
120
0
40
80
120
Trap height (cm)

Figure 3



a
a
b
500
400
300
200
100
0
Mean catch per trap
0m
20m
40m
Distance from field margin (m)

555 **Figure 4**



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